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DETERMINATION OF SELENIUM TOXICITY FOR SURVIVAL AND REPRODUCTION OF ENCHYTRAEID WORMS IN A SANDY LOAM SOIL

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14. ABSTRACT
We investigated toxicity of selenium (Se) to the soil invertebrate potworm *Enchytraeus crypticus* using the Enchytraeid Reproduction Test (ISO 16387; International Organization for Standardization; Geneva, Switzerland, 2004). Studies were designed to generate ecotoxicological benchmarks for developing the ecological soil screening levels (Eco-SSLs) for risk assessments of contaminated soils. For the present study, we selected aerobic upland soil Sassafras sandy loam (SSL), with soil characteristics (low levels of clay and organic matter, soil pH adjusted high) that support high relative bioavailability of the anionic Se species typically found in soil. Se was amended into SSL soil as sodium selenate. While under ambient greenhouse conditions, the amended SSL soil was subjected to weathering-and-aging by 21 d of alternating cycles of air-drying and rehydration to 60–75% of the soil water-holding capacity. Se concentrations that produced 20 and 50% decreases in reproduction for Se weathered-and-aged in SSL soil compared to control response (EC_{20} and EC_{50} values, respectively) were 4.4 and 6.2 mg/kg, respectively. Toxicity benchmarks established in the present study have been submitted to the U.S. Environmental Protection Agency Eco-SSL Workgroup, and the EC_{20} value was used in developing soil invertebrate-based Eco-SSL for Se.

15. SUBJECT TERMS

Ecological soil screening level (Eco-SSL)	Selenium	Natural soil
Enchytraeus crypticus	Selenate	Toxicity assessment
Sassafras sandy loam (SSL)	Selenite	

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PREFACE

The work described in this report was authorized under U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment (USEPA–ORD/NCEA), Interagency Agreement identification number DW-21-93926501-0. The work was started in July 2002 and completed in May 2006.

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DETERMINATION OF SELENIUM TOXICITY FOR SURVIVAL AND REPRODUCTION OF ENCHYTRAEID WORMS IN A SANDY LOAM SOIL

1. INTRODUCTION

Selenium (Se) occurs naturally in the environment due to weathering of rocks and volcanic activity, and consequently is released into the soil, water, and air. Se is also released anthropogenically from burning coal or oil. Se found in air is commonly attached to fly ash and suspended particles. Airborne selenium particles can settle on soil or water surfaces. Disposal of Se in commercial products and waste can also contribute to Se contaminant levels in soil. Se has been found in at least 508 of the 1623 current or former hazardous waste sites on the National Priorities List (Agency for Toxic Substances and Disease Registry [ATSDR], 2003). An average Se concentration in the earth's crust of 0.05–0.09 mg/kg was estimated by Glover et al. (1979). Se has been found in volcanic rocks in the western United States at concentrations up to and including 120 mg/kg (Glover et al., 1979) and in the vicinity of sandstone-type uranium deposits at concentrations as high as 1000 mg/kg (Shamberger, 1981). Most seleniferous soils contained <2 mg/kg, with a maximum Se concentration of <100 mg/kg reported by Rosenfeld and Beath (1964). Overexposure to Se can have detrimental effects on human and animal health (ATSDR, 2003).

Se can occur in the soil environment in the Se^{6+} (selenate), Se^{4+} (selenite), Se^{0} (elemental), and Se^{2-} (selenide) oxidation states, depending upon the redox (Eh) and pH values (Brown et al., 1999). Se is relatively insoluble and immobile in its reduced forms. However, in oxidized forms, particularly Se^{6+}, Se is mobile in aqueous solutions and poses a significant risk to organisms. Naturally occurring forms of Se include selenates [i.e., containing $(Se^{6+}O_4^{8-})^{2-}$], selenites [containing $(Se^{4+}O_3^{8-})^{2-}$], elemental Se (Se^{0}), and various metal selenides [e.g., berzelianite (Cu_2Se) or umangite (Cu_2Se_2)], and organic selenides.

Notwithstanding the evidence for Se persistence in soil, data in the published literature were insufficient to establish the screening level concentrations for assessment of ecological risks at Se-contaminated sites. Much of the previous research published on the ecotoxicology of Se has focused primarily on aquatic or wetland systems (Maier and Knight, 1994; USEPA, 1998; Sappington, 2002; Zawislanski and McGrath, 1998) or microbial transformations of Se (Losi and Frankenberger, 1998). Minimal information that is available on the effects of Se on terrestrial invertebrates was established in insect feeding studies (Hladun et al., 2012; Jensen and Trumble, 2003; Vickerman et al., 2004; Vickerman and Trumble, 1999). Even less information exists on the ecotoxicological effects of Se on soil invertebrates. Fischer and Koszorus (1992) investigated the sublethal effects of Se on the earthworm *Eisenia fetida*, but the exposures were conducted in a 1:1 (m/m) mixture of peaty marshland soil and horse manure, which is not representative of Se bioavailability conditions of upland aerobic soils. To fill the existing data gap, we conducted a definitive study designed specifically to meet the U.S. Environmental Protection Agency (USEPA) criteria for derivation of toxicity benchmarks acceptable for inclusion in the development of ecological soil screening levels (Eco-SSLs; USEPA, 2005) for Se released into upland aerobic soil environments. Eco-SSLs are concentrations of contaminants in soil that are protective of ecological receptors that commonly come into contact with soil or ingest biota that live in or on such soils. These values can be used

in the screening level ecological risk assessment (SLERA) to identify those contaminants that are not of potential ecological concern in soils and thus do not require further evaluation in the baseline ecological risk assessment (BERA). Exceeding an Eco-SSL value in a SLERA does not indicate that an environmental problem exists, but does indicate that further investigation in a BERA is merited. Eco-SSLs are consistent national screening values that can potentially result in cost savings during ecologically based site assessments and remedial investigations. Use of Eco-SSL values can help site managers distinguish sites that do not pose significant environmental risks from those that may pose risk, prioritize contaminated sites by the level of risk posed, quantify the relative risks at each site, and decide whether further investigation in a BERA is merited to determine appropriate remedial actions.

2. MATERIALS AND METHODS

2.1 Soil Collection and Characterization

We utilized natural soil, Sassafras sandy loam (SSL: fine-loamy, siliceous, semiactive, mesic Typic Hapludult; U.S. Department of Agriculture, 1975), collected from an open grassland field in the coastal plain on the property of the U.S. Army Aberdeen Proving Ground, Harford County, MD. This soil was selected for developing ecotoxicological values protective of soil organisms because (1) it was previously used to establish ecotoxicological benchmarks for organic and inorganic chemicals in standardized soil invertebrate toxicity tests (Kuperman et al., 2003, 2004a, 2004b, 2005, 2006a–2006d, 2012, 2013a, 2013b; Simini et al., 2003); and (2) it has physical and chemical characteristics that support qualitatively “high” relative bioavailability for metals in natural soils (USEPA, 2005). The pH of this slightly acidic soil was adjusted to represent conditions of an alkaline aerobic upland soil, to promote the formation of water-soluble forms of Se (Lemly, 1997), thus increasing the relative bioavailability of Se (USEPA, 2005) in toxicity tests.

During soil collection in the field, vegetation and the organic horizon were removed, and the top 12 cm of the A-horizon were then collected. The soil was sieved through a 5 mm screen, air-dried for at least 72 h, mixed periodically to ensure uniform drying, passed through a 2 mm sieve, and stored at room temperature before use in testing. The soil was then analyzed for physicochemical characteristics (Table 1). The water holding capacity (WHC) of SSL soil was determined to be 18% of the soil dry weight.

Table 1. Physical and Chemical Characteristics of Sassafras Sandy Loam (SSL) Soil Before and After Addition of CaO

Soil Parameter	SSL	SSL with 0.1% CaO
Sand (%)	70	70
Silt (%)	13	13
Clay (%)	17	17
Texture	Sandy loam	Sandy loam
Cation exchange capacity (cmol/kg)	5.49	9.8
Organic matter (%)	1.3	1.0
pH	5.2	7.1

2.2 Chemicals and Reagents

Calcium oxide (CaO; Chemical Abstracts Service [CAS] no. 1305-78-8; lot no. 4521MZ; purity, 98%; Aldrich Chemical Company; Milwaukee, WI) was used to raise the soil pH level to >7. Sodium selenate (anhydrous $H_2O_4Se{\cdot}2Na$; CAS no. 13410-01-0; lot no. G14105; purity, 99.8%; Alfa Aesar; Ward Hill, MA) was used to prepare soil treatment concentrations. Beryllium sulfate ($BeSO_4{\cdot}4H_2O$; CAS no. 7787; purity, 99.99%) was used as the positive control in definitive testing. ASTM Type I water (18 MΩ cm at 25 °C; ASTM, 2004b) was used throughout the toxicity studies. It was obtained using a Milli-RO 10 Plus followed by a Milli-Q PF Plus system (Millipore; Bedford, MA). The same grade of water was used throughout the analytical determinations. Glassware was washed with phosphate-free detergent and sequentially rinsed with tap water, ASTM Type II water (>5 MΩ cm at 25 °C), analytical reagent-grade nitric acid 1% (v/v), and ASTM Type I water.

2.3 Soil Preparation

In order to enhance Se bioavailability for this study, the SSL soil was amended with CaO to raise the pH level to >7. Aliquots of SSL soil were amended with different levels of CaO to determine the concentration needed to raise the soil pH from 5.3 to >7. The soil was moistened to initiate its reaction with CaO. The soil pH was monitored daily during the first week, then weekly. Results showed that 0.1% CaO was needed to raise the pH of the SSL soil to >7. Sufficient soil for range-finding and definitive studies was amended with 0.1% CaO. The soil stabilized at a mean pH of 7.1. This prepared soil was then used in toxicity tests to establish ecotoxicological benchmarks for the effects of Se on the test soil invertebrate species. Physicochemical properties of the SSL soil were again characterized following the addition of 0.1% CaO (Table 1).

Portions of air-dried SSL soil, pH 7.1, were prepared by mass for each Se treatment level and then amended with sodium selenate to establish target treatment concentrations. Aqueous (ASTM Type I water) stock solutions of 100 and 1000 mg/L sodium selenate were prepared. Appropriate aliquots of stock solutions were diluted with ASTM Type I water and then added to the soil to achieve target treatment concentrations of Se simultaneously hydrating the SSL soil to 17.1% dry weight (equivalent to 95% of the WHC of SSL soil). The

amended soil aliquots were mixed thoroughly and allowed to equilibrate for 24 h. Portions of these amended SSL soils were used in a range-finding test. The remainder of the amended soils were used in the weathering-and-aging process described in Section 2.4.

2.4 Weathering-and-Aging Se in Soil

Se treatments in SSL soil used in the definitive toxicity testing were subjected to a weathering-and-aging process before commencing tests in order to provide appropriate benchmark data for Eco-SSL development. Standardized methods for weathering-and-aging of chemicals in soil were not available. We have developed procedures that simulate, at least partially, the natural weathering-and-aging processes for chemicals in soil. These procedures allowed us to more-accurately approximate the exposure conditions for soil biota in the field, compared with tests conducted with freshly amended chemicals or tests conducted following a short equilibration period (e.g., 24 h) (Kuperman et al., 2004a, 2005, 2006b–2006d; Simini et al., 2003). Before definitive toxicity testing was conducted, samples of each freshly amended soil were initially rehydrated with ASTM Type I water to 60% of the WHC to initiate weathering-and-aging of Se in soil in open, chemically inert containers. The soil was then subjected to alternating hydrating and air-drying cycles at ambient temperatures in a greenhouse for 21 d. All soil treatments were reweighed and readjusted to their initial masses by addition of ASTM Type I water. Hydration frequency varied from one to two times each week, depending on the rate of soil drying. After completion of the Se weathering-and-aging procedures, all soil treatments were brought to 100% of the WHC of SSL soil 24 h before commencement of definitive toxicity testing.

2.5 Analytical Determination of Se in Soil

At the beginning and end of the test period (28 d), soil from each Se treatment level was collected, lyophilized, and stored at –40 °C. Three replicate soil samples (17.5 g each) were collected randomly from homogenized soil in each soil treatment level, including controls that did not receive Se. The soil was placed in 50 mL polypropylene centrifuge tubes with screw caps and frozen in a liquid nitrogen bath. After bubbling of the liquid nitrogen was reduced to a low simmer (approximately 15 min), the tubes were removed, and the screw caps were replaced by caps with vent holes. The soil was lyophilized for 24 to 48 h using a Labconco 6 L benchtop system (Labconco; Kansas City, MO). Vented caps were replaced by solid caps after lyophilization was complete. The soils were stored at –20 °C. Frozen samples were packed in dry ice in an insulated cooler and shipped overnight to Dr. Richard Higashi (who was at University of California–Davis at that time) for nitric–perchloric digestion and analytical determinations of Se and its speciation by liquid chromatography/inductively coupled argon plasma mass spectrometry. These determinations included total extractable Se (range-finding and definitive tests) and inorganic Se speciation analyses (range-finding tests only).

2.6 Toxicity Assessments

Several soil invertebrate toxicity tests, for which standardized protocols have been developed by the International Organization for Standardization (ISO, 1998a, 1998b), can effectively be used to assess toxicity and derive protective benchmark values (Stephenson et al., 2002; Løkke and Van Gestel, 1998). We adapted the ISO 16387 Enchytraeid Reproduction Test

(ISO, 2004) bioassay to assess the effects of Se on the potworm *Enchytraeus crypticus*. This test was selected on the basis of its use for measuring chemical toxicity to ecologically relevant test species during chronic assays, and also because of its inclusion of at least one reproduction component among the measurement endpoints. Our adaptation of ISO 16387 consisted of its use with natural soils and the enchytraeid worm *E. crypticus* as the test species. The ISO guideline for this assay was originally developed for use with Organisation for Economic Co-Operation and Development (OECD, 1984) artificial soil (a similar soil formulation was later adapted for USEPA standard artificial soil [USEPA, 1996] and for ASTM artificial soil [ASTM, 2004a]). However, several studies demonstrated that this test could also be conducted using natural soils (Amorim et al., 2005a, 2005b, 2009; Kuperman et al., 2003, 2004a, 2004b, 2005, 2006a–2006d). The ISO 16387 bioassay was initially developed using the enchytraeid worm species *Enchytraeus albidus*. Results of our previous studies using *E. albidus* showed that for optimal test conditions, this species requires soils containing high organic matter content with pH 6 (±0.5). *E. albidus* performed poorly in natural soils having physical and chemical characteristics that support relatively higher levels of bioavailability (Amorim et al., 2005a, 2005b, 2009; Kuperman et al., 1999, 2006a). *E. crypticus,* which is listed in the ISO protocol as an acceptable alternative to *E. albidus,* was therefore selected as the potworm species for toxicity testing.

Potworms were bred in 4.3 L clear plastic boxes (34 × 20 × 10 cm) filled with 2 kg (dry mass) of moist SSL soil. The culture was kept in an environment-controlled incubator using a 16 h light–8 h dark photoperiod cycle with a mean photosynthetically active radiation (PAR) light intensity of 12.8 ± 0.7 µM m^2/s (985 ± 52 lux), and mean temperature of 21.6 ± 0.1 °C. The soil moisture level was adjusted to 100% of the WHC of SSL soil, and it was maintained by periodic (once per week) mass checks and water adjustments. Soil in the breeding culture was aerated by carefully mixing it once each week. The potworms were fed approximately twice each week with ground oats spread onto the soil surface. If food from the previous feeding date remained on the soil surface, the amount of food added was adjusted. Every 6 weeks, the worms were transferred into a freshly prepared culture substrate. Cultures were synchronized so that all worms used in each toxicity test were approximately the same age. The potworm culture was deemed healthy if worms were whitish in color, reproduced continuously, did not try to leave the soil, and exhibited a shiny outer surface with no soil particles clinging to them.

Glass vessels (jars, 42 mm i.d. × 45 mm height) were used as test containers. Before the jars were used in the toxicity tests, they were cleaned with acetone, rinsed successively with tap water then ASTM Type I water, and then air-dried. Twenty grams (dry mass basis) of prepared Se treatment soil and 0.05 g of ground oats were added to each test container, mixed, and hydrated to 100% of the WHC of each soil. The mass of each container with hydrated soil was recorded.

Adult potworms with eggs in the clitellum region were used for testing. They were collected from culture and placed in a Petri dish filled with a small amount of ASTM Type I water for examination with a stereomicroscope. Potworms with no eggs were discarded; any invertebrates living in the cultures (e.g., mites) were also removed. Ten potworms, selected for uniformity (approximately 1 cm in length), were placed on top of the prepared hydrated treatment soil in each test container. Transparent plastic wrap was stretched over the top of each container and secured with a rubber band. Three pinholes were made in the plastic wrap to

facilitate air exchange. All test containers were placed in an environment-controlled incubator under a 16 h light–8 h dark photoperiod cycle with a mean PAR light intensity of 12.8 ± 0.7 µM m^2/s (985 ± 52 lux), and a mean temperature of 21.6 ± 0.1 °C, for the duration of the 28 d test. The containers were reweighed once each week, and the mass loss was replenished with the appropriate amount of ASTM Type I water. At those times, 0.05 g of ground oats were added atop the soil within each test container.

After 14 d, soil in each test container was carefully searched, and adult potworms were removed and counted. Potworms were examined for any morphological or behavioral changes. The remaining test substrate, including any cocoons laid during the first 2 weeks of the test, was incubated for additional 14 d. After 28 d from the start of the test, soil in the test containers was fixed with 70% ethanol, and 9 drops of rose bengal biological stain (1% solution in ethanol) were added. Staining continued for at least 24 h. The content of each test container was then wet-sieved using a 100 mesh (150 µm) sieve, and retained contents were transferred to a counting tray, where potworms were counted. Measurement endpoints included the number of adults surviving after 14 d and the number of juveniles produced after 28 d.

Treatment Se concentrations for definitive testing were selected based on the results of the range-finding test, which was conducted to bracket 20 and 50% inhibition in juvenile production, compared with juvenile production in negative-control soil. Definitive testing included the following replicated treatments: Se treatments (CaO-treated SSL soil with Se added), negative control (CaO-treated SSL soil with no Se added), pH control (SSL soil with no CaO or Se added), and positive control (toxicity tests with a reference toxicant). The positive-control treatment was prepared using a solution of beryllium sulfate in ASTM Type I water to attain 30 mg/kg Be nominal concentration in SSL soil (with no CaO added). The effects of the reference toxicant were determined by comparing the results obtained in Be treatment of SSL soil with results of pH control treatment. Validity criteria for the negative-control treatment included the following performance parameters (ISO, 2004):

- The adult mortality does not exceed 20% after 14 d,
- The average number of juvenile potworms per test container at the end of the test is greater than 2.5-fold the initial number of adult potworms per test container, and
- The coefficient of variation for the mean number of juveniles is ≤50%.

2.7 Data Analyses

Data for production of juveniles were analyzed using regression models selected from among those described in an Environment Canada (EC) guidance document (EC, 2005). During the statistical model selection process, compliance with the normality assumptions and homoscedasticity of the residuals were determined by examining the stem-and-leaf graphs and histograms of the residuals. The best fit to data was evident when the regression lines generated by the models were closest to the data points; the regression coefficients for point estimates were the greatest; the residuals were homoscedastic (i.e., had the most random scattering); and the

means, standard errors, and variances of the residuals were the smallest. The logistic Gompertz model is described by

$$Y = a \times \mathrm{e}^{\{[\log(1-p)]\times(\mathrm{C}\div\mathrm{ECp})^{b}\}}$$

where

- Y is the dependent variable for a measurement endpoint (e.g., the number of juveniles or adults);
- a is the y-axis intercept (i.e., the control response);
- e is the exponent of the base of the natural logarithm;
- p is the desired value for "p" effect (e.g., 0.50 for a 50% decrease from the control response, EC_{50});
- C is the exposure concentration in the test soil;
- ECp is the estimate of concentration for a specified percent effect; and
- b is a scale parameter that defines the shape of the equation.

This logistic Gompertz model was selected for the analysis of juvenile production data determined in the definitive test. The ECp parameters used in the present study included the Se concentration producing a 20% (EC_{20}) or 50% (EC_{50}) decrease in the measurement endpoint compared with the negative control. The EC_{20} parameter based on a reproduction endpoint is the preferred parameter for deriving Eco-SSL values. The EC_{50} parameter, a commonly reported value, was included to enable comparisons of the results produced in this study with results reported by other researchers. The 95% confidence intervals (CIs) associated with the point estimates were determined.

Analysis of variance of data was used to determine the bounded (when possible) no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC) values for adult survival and juvenile production, respectively. Mean separations were done using Fisher's least-significant difference pairwise comparison tests. All statistical analyses were performed using untransformed data and analytically determined total extractable Se concentrations. A significance level of $p \leq 0.05$ (95% confidence level) was accepted for all statistical tests. Statistical analyses were performed using SYSTAT 11.0 (Systat Software, Inc.; Chicago, IL).

3. RESULTS

3.1 Range-Finding Test

A range-finding test was conducted to determine the range of Se concentrations in soil from a no-observed-effect level to a level of >50% reduction in production of juveniles by *E. crypticus*. Soil from the range-finding test was analyzed for total extractable Se, selenate [$(SeO_4)^{2-}$], and selenite [$(SeO_3)^{2-}$]. Results of chemical analyses are shown in Table 2. Selenate accounted for 25 to 86% of the extractable Se at the start of the experiment and for 7 to 65% of the extractable Se at the end of the experiment. The percentage of Se as selenate increased with Se treatment concentration (Table 2). Concentrations of selenite were below levels of detection at both the start and end of the experiment (Table 2). Therefore, selenate was considered to be the principle available form of Se under these experimental conditions.

Table 2. Nominal and Measured Se Concentrations in Soil at the Start and End of the Range-Finding Toxicity Test with *E. crypticus*

Nominal Se Concentration (mg/kg)	Mean Se Concentration in Soil (mg/kg)						Soil pH (at Start)
	Start			End			
	Se Total	Selenate	Selenite	Se Total	Selenate	Selenite	
0	0.16	0.004	BDL	0.90	BDL	BDL	7.07
0.1	0.20	0.05	BDL	0.54	0.04	BDL	7.08
1	0.67	0.39	BDL	1.54	0.35	BDL	7.08
10	5.56	4.11	BDL	8.84	4.25	BDL	7.09
100	74.07	45.78	BDL	100.25	44.76	BDL	6.89
1000	740.75	635.90	BDL	876.79	568.91	BDL	6.70

Note: Se concentrations included total extractable (Se total), selenate, and selenite fractions. The pH values measured at the initiation of the bioassay experiments were determined after the Se treatment additions as selenate. BDL, below detection limit.

These results indicate that available Se was primarily in the form of selenate in the present study, as would be expected in soils sustaining high relative bioavailability of Se. Results of the range-finding test for the toxicity of Se in soil to the potworm *E. crypticus* are presented in Table 3. Adult survival and reproduction were not affected in soil Se concentrations up to and including 0.67 mg/kg, measured at the start of the test. At a Se concentration of 5.56 mg/kg, survival of adult *E. crypticus* decreased 11% as compared with the negative-control treatment (Table 3). Production of juveniles by *E. crypticus* was decreased by 99.2% at a Se concentration of 5.56 mg/kg.

Table 3. Effects of Se in Soil (Initially Added into Soil as Selenate) on *E. crypticus* in Range-Finding Toxicity Testing

Measured Se Concentration (mg/kg)	Adults Surviving, Mean (SE)	Juveniles Produced, Mean (SE)
0.16*	9.5 (0.5)	1764 (66)
0.20	9.5 (0.5)	2033 (201)
0.67	10.0 (0.0)	1903 (113)
5.56	8.5 (1.5)	13.5 (10.5)
74.1	0	0
741	0	0

*Analytically determined background concentration of Se in SSL soil used in range-finding toxicity testing.
SE, standard error.

3.2 Definitive Test

Definitive testing using the adapted ISO 16387 Enchytraeid Reproduction Test (ISO, 2004) was conducted to assess the acute (adult mortality) and chronic (juvenile production) effects of Se on *E. crypticus* in SSL soil. Measurement endpoints were assessed using treatment

concentrations that were based on the results of the range-finding studies. Measurement endpoints included the number of surviving adults after 14 d and the number of juveniles produced after 28 d of exposure, respectively, to Se treatments. Exposure concentrations were selected for definitive testing to achieve bracketing of significant effects on the reproduction endpoint (i.e., production of juveniles). Reproduction endpoints are preferred for the development of Eco-SSL values for soil invertebrates (USEPA, 2005) and were therefore the main focus of the present study. All ecotoxicological parameters were estimated using these measurement endpoint values and analytically determined concentrations of Se in soil.

Test results complied with the validity criteria defined in the ISO 16387 (2004) test guideline, including those mentioned in Section 2.6 of this report. The validity criteria for test results from the negative-control treatment included a 98% mean adult survival, a mean number of 1430 juveniles produced, and a 13% coefficient of variation. Juvenile production in the positive-control treatment was decreased by 60% as compared with the pH control (SSL soil with no CaO added) and was within the baseline established for the laboratory culture of *E. crypticus* (Kuperman et al., 2004b). Test compliance with the validity criteria confirmed that the toxicological effects determined in the definitive testing were attributable to the Se treatments.

Results of definitive testing are shown in Table 4, and ecotoxicological responses of *E. crypticus* to Se are summarized in Table 5. Both adult survival and juvenile production were affected in Se-amended soils within the concentration ranges selected for definitive testing. Juvenile production was the more-sensitive measurement endpoint for assessing Se toxicity to *E. crypticus* as compared with adult survival, which comports with results of our previous studies.

Table 4. Effects of Se Weathered-and-Aged in SSL Soil on Toxicity to *E. crypticus*, Determined in Definitive Toxicity Testing

Nominal Se Concentration (mg/kg)	Measured Se Concentration† (mg/kg)	Adults Surviving, Mean (SE)	Juveniles Produced, Mean (SE)
0 (pH control)‡	NA	9.75 (0.25)	1440 (97)
0 (negative control)	0.35	9.75 (0.25)	2164 (28)
1	1.02	10.0 (0.0)	2148 (59)
2	2.32	10.0 (0.0)	2033 (41)
4	4.03	10.0 (0.0)	1748 (39)
6	5.83	9.75 (0.25)	1287 (64)
8	9.01	9.5 (0.29)	199 (34)
12	11.35	6.5 (0.96)	0.3 (0.3)
Positive control (Be, 30 mg/kg)	NA	9.25 (0.48)	575 (42)

†Total extractable concentrations of Se initially added as sodium selenate.
‡SSL soil with no CaO or Se added.
NA, not applicable.
SE, standard error.

Table 5. Ecotoxicological Benchmarks for Adult Survival and Juvenile Production by *E. crypticus* Exposed to Se Weathered-and-Aged in SSL Soil

Ecotoxicological Parameter	Adult Survival (mg/kg)	Juvenile Production (mg/kg)
NOEC	9.01	1.02
p	0.664	0.788
LOEC	11.35	2.32
p	<0.0001	0.045
EC_{20}	ND	4.4
CI (95%)	ND	4.09–4.68
EC_{50}	ND	6.2
CI (95%)	ND	6.02–6.48
R^2	ND	0.997

p, probability value.
ND, not determined; could not be estimated within the concentration range tested.
R^2, coefficient of determination (regression coefficient).

The numbers of surviving adult *E. crypticus* were not significantly ($p = 0.664$) different between the negative control and the 9.01 mg/kg (bounded NOEC) treatments. Adult survival was significantly ($p < 0.0001$) decreased at the 11.35 mg/kg (bounded LOEC) treatment as compared with the negative control.

Juvenile production was significantly ($p < 0.045$) decreased in the 2.32 mg/kg concentration treatment as compared with the number of juveniles in the negative control, producing the bounded NOEC and LOEC values of 1.02 and 2.32 mg/kg, respectively (Table 5). The range of Se concentrations selected for the definitive test was sufficient to establish the concentration-response relationship based on juvenile production by *E. crypticus*, and the logistic Gompertz model had the best fit to the data (Figure 1). The regression coefficient (R^2) value determined for the juvenile production toxicity endpoint was 0.997, indicating a very good fit of the model to the toxicity data. Nonlinear regression analysis of toxicity data by the logistic Gompertz model yielded the EC_{20} and EC_{50} values and corresponding 95% CI for juvenile production of 4.4 (4.09–4.68) and 6.2 (6.02–6.48) mg/kg, respectively (Table 5).

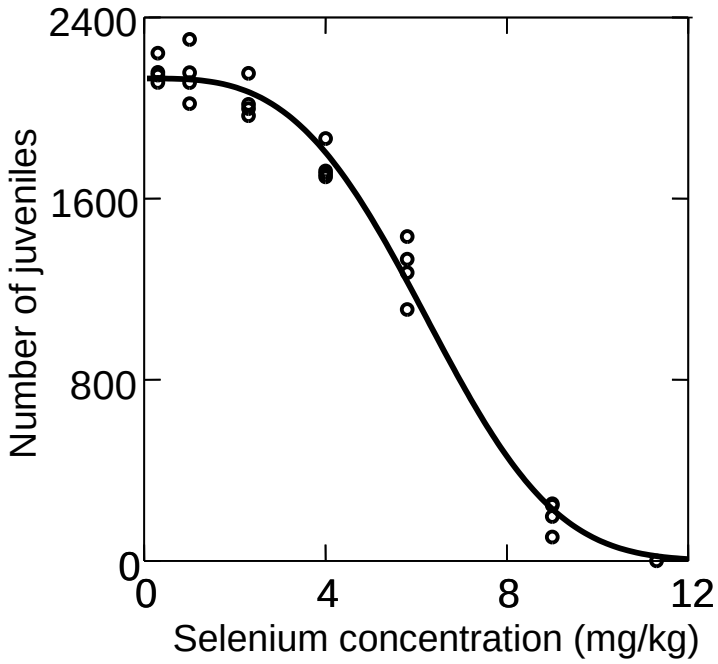

Figure 1. Effect of Se weathered-and-aged in SSL soil on the production of juveniles by *E. crypticus.*

4. DISCUSSION AND CONCLUSIONS

The present studies were designed to develop scientifically defensible toxicity data that are required for successful management of contaminated sites and knowledge-based decision-making. Generating toxicity data to establish a benchmark that is appropriate for utilization in deriving the soil invertebrate-based Eco-SSL values for Se was the main objective of this investigation. Ecotoxicological testing was specifically designed to successfully meet the criteria for Eco-SSL derivation outlined in the Eco-SSL guideline (USEPA, 2005). Eco-SSL test acceptance criteria were met or exceeded in this investigation by ensuring that the following occurred:

(1) Tests were conducted in natural soil having physicochemical characteristics that support high relative bioavailability of Se,
(2) Experimental designs for laboratory studies were documented and appropriate,
(3) Both nominal and analytically determined concentrations of chemicals of interest were reported,
(4) Tests included both negative and positive controls,
(5) A chronic or life cycle test was used,
(6) Appropriate chemical dosing procedures were reported,
(7) Concentration–response relationships were reported,

(8) Statistical tests used to calculate the benchmarks and levels of significance were described, and
(9) The origin of the test species was specified and appropriate.

The preferences for reproduction benchmarks and a relatively low effect level (i.e., EC_{20}) were justified, to ensure that Eco-SSL values would be protective of populations of the majority of ecological receptors in soil, and provide confidence that Se concentrations posing an unacceptable risk are not screened out early in the ecological risk assessment process during the SLERA. The exposure concentrations of Se in soil were analytically determined at the beginning of the definitive toxicity testing; consequently, the ecotoxicological benchmarks were determined using measured Se concentrations. This complied with the USEPA preference for derivation of Eco-SSL values on the basis of measured concentrations of a chemical in soil, over those based on nominal concentrations (USEPA, 2005).

The present studies included weathering-and-aging of Se in soil within the experimental design to produce a soil microenvironment more similar to field conditions, and thus more closely approximate the exposure effects in contaminated sites. Therefore, toxicity benchmarks generated in these studies contributed to development of an Eco-SSL value for Se that better represents the exposure conditions of soil invertebrates at contaminated sites.

Definitive testing in these studies using exposures of the potworm *E. crypticus* in upland aerobic sandy loam soil has established new ecotoxicological data for Se effects on soil invertebrates under conditions of high relative bioavailability of Se in soil, as defined by the USEPA (2005). The EC_{20} and EC_{50} values based on the toxicity of Se to *E. crypticus* reproduction (Table 5) were remarkably similar to those established for other soil invertebrates, including the earthworm *Eisenia andrei* (3.4 and 3.9 mg/kg, respectfully) and the collembolan *Folsomia candida* (4.7 and 10.9 mg/kg, respectfully), which are commonly used in standardized toxicity tests (USEPA, 2007). EC_{20} benchmarks based on reproduction endpoints are preferred for deriving Eco-SSLs for soil invertebrates (USEPA, 2005). The EC_{50} value is provided for relative comparison with the EC_{50} values for other anionic contaminants, since EC_{50} values are more commonly reported in the literature. The results of the present research, along with the Se benchmarks developed for *E. andrei* and *F. candida* in our other separate studies (Kuperman et al., 2016), were submitted to the Eco-SSL National Task Group for quality-control review. Subsequently, these toxicity data were included in the Eco-SSL database and used in developing a Se Eco-SSL of 4.1 mg/kg for soil invertebrates (USEPA, 2007). This Eco-SSL value for Se is comparable with the Canadian Se environmental soil-quality guideline values of 1 mg/kg for agricultural, residential, and parkland land uses, and 2.9 mg/kg for commercial and industrial land uses (CCME, 2009).

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ABBREVIATIONS AND ACRONYMS

ATSDR	Agency for Toxic Substances and Disease Registry
BDL	below detection limit
BERA	baseline ecological risk assessment
CAS	Chemical Abstracts Service
CCME	Canadian Council of Ministers of the Environment
CI	confidence interval
EC	Environment Canada
EC_{20}	Concentration producing a 20% decrease in the measurement endpoint
EC_{50}	Concentration producing a 50% decrease in the measurement endpoint
ECBC	U.S. Army Edgewood Chemical Biological Center
Eco-SSL	ecological soil screening level
ECp	Effect concentration for a specified percent effect
ISO	International Organization for Standardization
LOEC	lowest-observed-effect concentration
NA	not applicable
ND	not determined
NOEC	no-observed-effect concentration
OECD	Organisation for Economic Co-Operation and Development
p	probability value
PAR	photosynthetically active radiation
R^2	coefficient of determination (regression coefficient)
SE	standard error
SLERA	screening level ecological risk assessment
SSL	Sassafras sandy loam
USEPA	U.S. Environmental Protection Agency
WHC	water-holding capacity

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